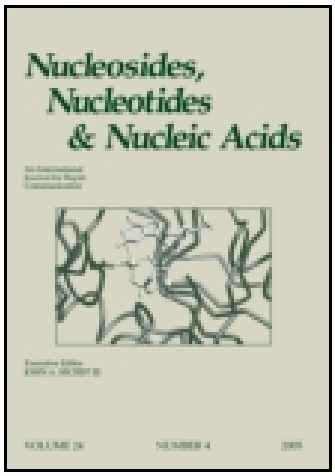

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OXIDATION OF BIOTIN DURING OLIGONUCLEOTIDE SYNTHESIS

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▫ *A new "polystyrene biotin support" has been synthesized for the solid support synthesis of the 3′-biotinylated oligonucleotides. Several oligos were synthesized and were analyzed by the HPLC and Mass Spec. Oligo analysis revealed that the biotin gets oxidized to "biotin sulfoxide" during the synthesis.*

Keywords Biotin, Oligonucleotide Synthesis

INTRODUCTION

The strong affinity between biotin and streptavidin or avidin ($K_d = 10^{-15}$/M) is exploited in many molecular biology applications.[1] Biotin can be conjugated to oligonucleotide at the 5′-end, at the 3′-end or internally depending on the application. For the 3′-end biotin labeling of the oligo, commercially available, biotin controlled pore glass (CPG) support is used.[2] Compared to CPG support, polystyrene has special advantages.[3] Applied biosystems has developed a high-throughput DNA synthesizer 3900™, which exclusively uses the polystyrene support (PS) for the high throughput DNA synthesis. To facilitate, the synthesis of a high throughput 3′-biotin labeled oligonucleotides, there was a need for polystyrene biotin support. Here in we report the synthesis of the "biotin polystyrene support," and its use in the oligonucleotide synthesis. We also report here, for the first time, the oxidation of biotin, to biotin sulfoxide, during the oligosynthesis.

SYNTHESIS OF BIOTIN SUPPORT

Biotin **1** was suspended in pyridine (Scheme 1) and was reacted with t-butylchlorodiphenylsilane and t-butylbenzoylchloride sequentially to convert to N-t-butylbenzoylbiotin **2**.[4] The carboxyl group in compound **2** was activated and coupled with 1-O-DMT-2-aminobutyl-1, 3-propane diol linker **3**,[3] to yield compound **4**. The hydroxyl group in compound **4** was reacted with succinic

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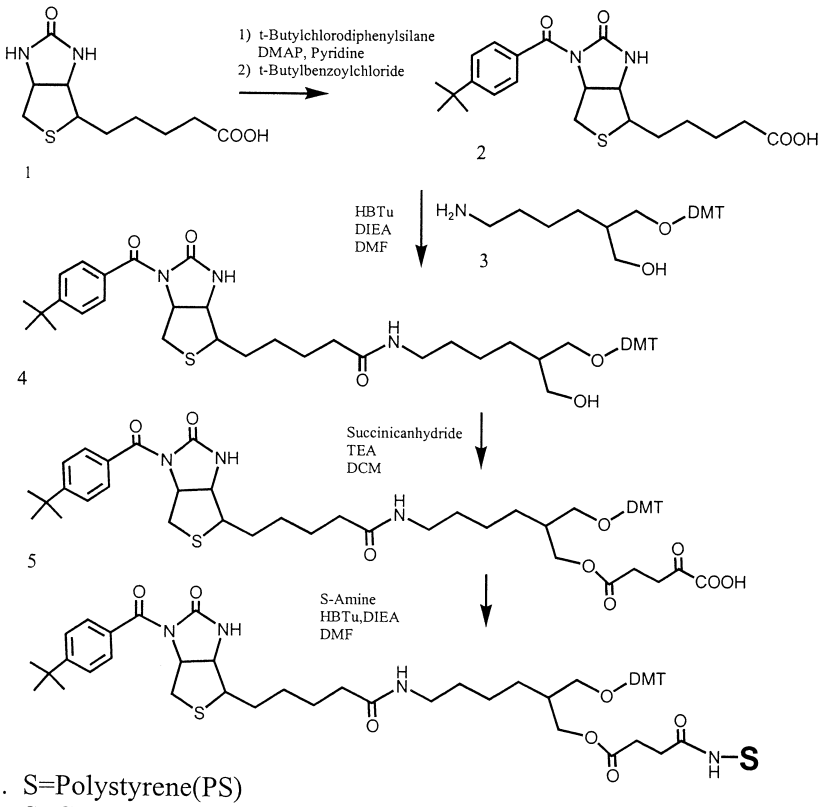

SCHEME 1 Synthesis of biotin support.

anhydride to get compound ***5***. The carboxyl group in compound ***5*** was activated and coupled to the amine functionalized support to yield biotin-support **6** and **7**. Any uncoupled, free amines were capped with acetyl groups.

3′-BIOTINYLATED OLIGONUCLEOTIDE SYNTHESIS

Standard columns were packed with 0.2 μmol biotin PS support or with 0.2 μmol biotin CPG support. Oligonucleotides were synthesized either on ABI

TABLE 1 Representative Biotinylated Oligonucleotides Synthesized

TTT TTT TTT TTT TTT TT-Biotin-3′
TGG ATAGCG ACG TGG-Biotin-3′
ATC ACT GGA TAG CGA GGT GG-Biotin-3′
CCC CAA TCA CTG GAT AGC GAC GTGG-Biotin-3′
CCC ACC CCC AAT CAC TGG ATA GCG ACG TGG-Biotin-3′
ATC AGC CCC ACC CCC AAT CAC TGG ATA GCG ACG TGG-Biotin-3′

394™ or on ABI 3900™ DNA synthesizer, following standard cycles. On completion of the synthesis, oligonucleotides were cleaved and deprotected manually, by heating the "support" in concentrated ammonium hydroxide, at 65°C for 3 h in a sealed vial. Oligos were analyzed and purified by HPLC and characterized by MS (Table 1).

RESULTS AND DISCUSSION

HPLC analysis of the oligos (Figure 1) synthesized on ABI 3900™ from the biotin-PS support showed two major products. They were separated and purified by HPLC and analyzed by mass spectrometry. Interestingly, the mass difference between the two products was 16 Dalton units, which accounts for one oxygen atom. A plausible explanation is that the sulfur atom in the biotin gets oxidized to sulfoxide by the oxidizing reagent, iodine/pyridine/water (bottle 15), used in each cycle of the DNA synthesis. The oligos made on ABI 394™ by CPG support yielded only the oxidized product. Commercial CPG support also yielded biotin oxidized oligos. The amount of oxidized product depends on the length of the oligo and the nature of the support used.

Reduced concentration of the iodine reagent did not have significant effect on the oxidation. We also tried other oxidizing agents compatible with the DNA synthesis protocols either to selectively curb the biotin oxidation or to oxidize completely to the biotin sulfoxide. Replacing the iodine reagent with 10-camphor-sulfonyl-oxaziridine (CSO) didn't change the oxidation pattern. But the oxidizing agent, t-butylhydroperoxide (TBHP) completely oxidizes the biotin made from PS support to sulfoxide level, and the one made from biotin CPG support to a mixure of sulfoxides and sulfone.

To our knowledge, this phenomenon of biotin oxidation during the oligonucleotide synthesis mediated by the biotin support is not reported in the literature. One reason would be that for many applications, biotin is labeled at the 5′-end of

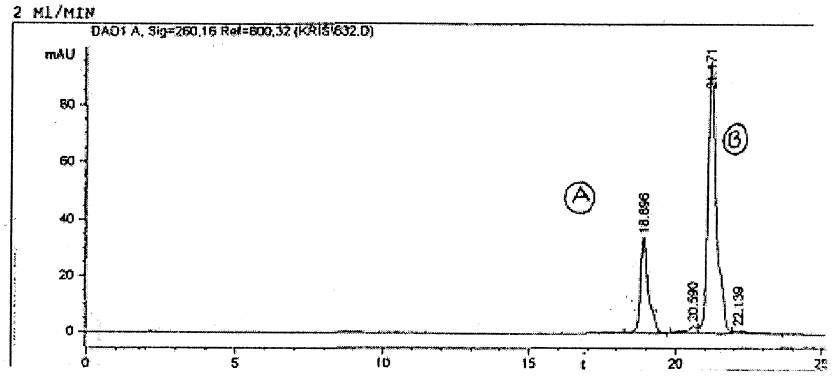

FIGURE 1 HPLC of the oligo 1. A) oxidized, B) expected.

the oligo and therefore it is not subjected to the repeated oxidation cycles, yielding the desired product. However in some other unrelated studies, the oxidation of biotin to biotin sulfoxide, and it's binding to avidin has been reported in the literatures.[5,6] The study showed that oxidation did not significantly alter the affinity toward avidin.

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